UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

FEB 20 1990

Subject: PP#8F3603, Supplemental Submission on Pyridate; Feeding Study in the Lactating Cow, Feeding Study in the Laying Hen (MRID Nos. 413546-01, 413546-02, DEB Nos. 6264, 6265, HED Project No. 0-0592)

From: Elizabeth T. Haeberer, Chemist
Tolerance Petition Section I
Dietary Exposure Branch
Health Effects Division (H7509C)

Thru: Richard D. Schmitt, Ph.D., Chief
Dietary Exposure Branch
Health Effects Division (H7509C)

To: Robert Taylor, PM 25
Herbicides and Fungicides Branch
Registration Division (H7505C)
and
Toxicology Branch
Health Effects Division (H7509C)

Agrolinz Incorporated has submitted two feeding studies for review, "Feeding Study in the Lactating Cow" (MRID No. 413546-01) and "Feeding Study in the Laying Hen" (MRID No. 413546-02).

No permanent tolerances have been established for residues of pyridate. DEB has recommended as follows for PP#8F3603, Pyridate on Cabbage, Corn and Peanuts, December 14, 1989, Elizabeth T. Haeberer:

> Due to the deficiencies in the Product Chemistry data (Conclusion 8), the need for submission of the modified analytical method (Conclusion 4a), and a revised Section B (Conclusions 6a and 6b), DEB can not recommend in favor of the proposed permanent tolerances at this time. DEB could recommend for establishment of the proposed tolerances, with an expiration date not to exceed one year, when the modified

analytical method and the revised Section B are provided. This would allow sufficient time for submission of the additional Product Chemistry data. The expression of tolerance would be as follows:

> Tolerances are established for the combined residues of the herbicide pyridate [O-(6-chloro-3-phenyl-4-pyridazinyl)-S-octyl-carbonothioate], the metabolite 6-chloro-3-phenyl-pyridazine-4-ol and conjugates of 6-chloro-3-phenyl-pyridazine-4-ol, expressed as pyridate in the following raw agricultural commodities:
>
> | | |
> |---|---|
> | Cabbage | 0.03 ppm |
> | Corn; grain, forage, silage, fodder | 0.03 ppm |
> | Peanuts; nutmeat, hulls | 0.03 ppm |

The following Conclusions and Recommendations do not apply to our review of PP#8F3603; they do not alter our previous conclusions regarding animal metabolism and feeding studies made in the DEB memo of 12/14/89. The following Conclusions and Recommendations apply only to future pyridate submissions involving livestock feed items bearing higher residues than the feed item residue levels considered in PP#8F3603.

CONCLUSIONS

1. In the DEB review of December 14, 1989, we determined that the nature of the residue in lactating ruminants was adequately defined and that the residue of concern was pyridate, its metabolite CL 9673 and its conjugates. The data presented in this submission raise some concerns regarding the nature of the residue in lactating ruminants. The questions raised in Conclusion 5, below, will have to be resolved.

2. As stated in the December 14, 1989 review, the nature of the residue in poultry is not adequately defined. If the petitioner proposes a new use for pyridate which could result in higher residues in animal feed items, a poultry metabolism study will be needed to characterize the nature of the residue.

3. The petitioner has based the 1, 3, and 10X feeding levels in both feeding studies on maximum residues of 1 ppm pyridate in/on alfalfa. The Agency currently does not have a submission for the proposed use of pyridate on alfalfa, therefore we can draw no conclusions concerning the adequacy of the feeding levels.

4. ^{14}C-Pyridate was used in both feeding studies,in order to facilitate qualitative and quantitative determination of pyridate residues. If the petitioner anticipates future uses

of pyridate which will lead to residues higher than the 0.03 ppm level proposed for cabbage, corn and peanuts, and a greater potential for secondary residues that will livestock commodity tolerances, a "cold" analytical method will be needed for the analysis of residues in meat, milk, poultry and eggs suitable for enforcement purposes.

5. The feeding study on lactating cattle is inadequate. The residues which have been identified must be quantitated. In addition, data are needed on the nature of the unidentified peaks, i.e., whether these peaks represent single compounds, and the percent of the total residue which is unidentified.

6. The feeding study on laying hens is inadequate. The residues which have been identified must be quantitated. In addition, data are needed on the nature of the unidentified peaks, i.e., whether these peaks represent single compounds, and the percent of the total residue which is unidentified. The nature of the residue in poultry remains inadequately defined.

RECOMMENDATIONS

The feeding studies in lactating ruminants and laying hens are inadequate for the reasons cited in Conclusions 1 through 6.

DETAILED CONSIDERATIONS

Although animal feeding studies were not needed for the DEB recommendation for establishment of tolerances proposed for cabbage, corn and peanuts at 0.03 ppm due to low residue levels in the subject crops at harvest, such data would be needed if future uses of pyridate resulted in higher residues in animal feed items. The residue of concern in lactating ruminants was previously defined as pyridate, its metabolite CL 9673 and its conjugates (see Conclusion 1 above). The nature of the residue in poultry and eggs is not adequately defined. (See review of December 14, 1989 cited above.) If future uses result in significant residues in poultry feed items, additional poultry metabolism data will be needed, fully characterizing the nature of the residue.

The current submissions are discussed in turn below.

FEEDING STUDY IN THE LACTATING COW

Twelve lactating Holstein cows were separated into four study groups of three cows each. During the experimental period the

animals were housed singly at all times. Food concentrate was allowed twice daily immediately after dose administration. Hay and water was allowed ad libitum. Each cow was implanted with an indwelling rumenal cannula to facilitate dose administration.

[^{14}C]-Pyridate (purity >9[illegible]%) was formulated as a solution in corn oil. Radiolabelled pyridate was used to quantify the total levels of pyridate related residues in plasma, tissues and milk, and to facilitate evaluation and characterization of tissue metabolites. The cows were sacrificed 6 hours after the final dose was administered. According to the petitioner the 28 day, twice daily dosing regime was designed to simulate feeding the dose levels 1, 3, and 10X the expected maximum residue level in feed, i.e., 1, 3.3, and 10 ppm based on 1 ppm in alfalfa. The Agency does not, at this time, have a petition under review for the use of pyridate on alfalfa, therefore we can draw no conclusions as to the adequacy of the feeding levels. In addition, if the petitioner anticipates future uses of pyridate which will lead to residues higher than the 0.03 ppm level proposed for cabbage, corn and peanuts, and a greater potential for secondary residues, a "cold" analytical method is needed for the analysis of residues in meat and milk which is suitable for enforcement purposes.

Radioactivity was quantitated using a liquid scintillation analyzer. The limit of reliability was determined to be 30 d.p.m. above background. The radioactivity on TLC plates was quantitated using a radio-TLC analyzer. Acid treated extracts were also analyzed by high performance liquid chromatography (HPLC) with a UV detector. Data from the detector was collected and analyzed using a Trivector Trio data station. Fractions were collected directly into scintillation vials and the levels of total radioactivity were measured in each fraction collected, by liquid scintillation counting.

Plasma levels of total radioactivity increased in proportion to increasing dose. Levels of radioactivity were steady after the first 2-3 days of administration. The following table compares total radioactive residues found in meat and milk at the 1 (1X), 3.3 (3X), and 10 ppm (10X) feeding levels. All control samples were below detection level.

SAMPLE	RESIDUES FOUND (PPM) FEEDING LEVELS		
	1X	3X	10X
Liver	0.018-0.021	0.062-0.226	0.18-0.22
Kidney	0.135-0.237	0.483-0.673	1.34-2.28
Heart	0.007-0.011	0.030-0.040	0.05-0.12
Lung	0.008-0.009	0.028-0.036	0.06-0.09
Brain	0.001	0.003-0.007	0.01-0.02
Muscle(Dorsal)	0.002-0.007	0.007-0.009	0.02-0.04
Muscle(Rump)	0.002-0.003	0.007-0.010	0.02-0.03
Muscle(Should.)	0.001-0.004	0.006-0.009	0.02-0.03
Subcut. Fat	0.002-0.004	0.008-0.032	0.01-0.02
Renal Fat	0.002-0.013	0.004-0.028	0.00-0.01
Bile	0.036-0.067	0.156-0.236	0.56-0.78
Whole Blood	0.011-0.014	0.044-0.051	0.10-0.13
Plasma	0.016-0.020	0.063-0.071	0.15-0.20
Bladder Urine	1.899-2.036	4.199-7.282	15.81-24.91
Milk	0.003-0.004	0.011-0.019	0.02-0.04

The data indicate that detectable residues would occur in liver, kidney and heart at the 1 ppm feeding level. At the 3.3 ppm feeding level residues >0.01 ppm would also occur in muscle and milk. At the 10 ppm feeding level all of the tissues tested had residue levels greater than 0.01 ppm.

Copies of the radio-HPLC profiles for the sample extracts have been submitted. The following compound peaks are identified from these curves: pyridate, CL 9673, CL 9673-N-glucuronide and CL 9673-O-glucuronide. Numerous additional peaks appear which have not been identified. The petitioner has not quantitated the known compounds, therefore no conclusions can be drawn concerning the residues found in lactating cattle from this feeding study. The petitioner must quantitate the levels at which the various residue components are present. A determination should be made of the percent of the total residue represented by the unidentified peaks. If these unknown peaks represent single compounds and are of significant quantity, they will have to be identified.

The feeding study on lactating cattle is inadequate. The residues which have been identified must be quantitated. In addition, data are needed on the nature of the unidentified peaks, i.e., whether these peaks represent single compounds, and the percent of the total residue which is unidentified.

FEEDING STUDY IN THE LAYING HEN

Forty young adult laying hens were separated into 4 groups of 10 hens each. Each hen was housed separately throughout the study. Food (556 Farmgate Layers Pellets) and water were available *ad libitum*. Group 1 was the control. Groups 2-4 were dosed twice daily, for 28 days, by gavage into the crop. ^{14}C-Pyridate was

formulated as a solution in corn oil and administered at the following dose levels: Group 2 - 0.1 mg/kg/day (1.3 ppm diet); Group 3 - 0.3 mg/kg/day (4 ppm diet); Group 4 - 1 mg/kg/day (13 ppm diet). The hens were sacrificed 6 hours after the last dose was administered.

Radioactivity was quantitated using a liquid scintillation analyzer. The limit of reliability was determined to be 30 d.p.m. above background. The radioactivity on TLC plates was quantitated using a radio-TLC analyzer. Acid treated extracts were also analyzed by high performance liquid chromatography (HPLC) with a UV detector. Data from the detector was collected and analyzed using a Trivector Trio data station. Fractions were collected directly into scintillation vials and the levels of total radioactivity were measured in each fraction collected, by liquid scintillation counting.

The following table compares total radioactive residues found in poultry and eggs at the 1.3 (1X), 4 (3X), and 13 ppm (10X) feeding levels (based on a 1 ppm residue level in alfalfa and adjusted for expected daily food consumption). All control tissues had residues below the level of reliable detection.

SAMPLE	RESIDUES FOUND (PPM) FEEDING LEVELS		
	1X	3X	10X
Liver	0.009-0.049	0.031-0.114	0.045-0.205
Kidney	0.011-0.075	0.080-0.277	0.061-0.510
Heart	0.003-0.032	0.010-0.056	0.000-0.078
Leg Muscle	0.001-0.009	0.002-0.020	0.002-0.026
Breast Mus.	0.001-0.010	0.000-0.015	0.001-0.017
Fat Pad	0.000-0.010	0.000-0.007	0.000-0.040
Skin+Fat	0.001-0.021	0.009-0.037	0.028-0.142
Plasma	0.005-0.071	0.026-0.140	0.065-0.210
Whole Blood	0.006-0.051	0.023-0.102	0.016-0.128
Egg White*	0.002-0.008	0.007-0.011	0.018-0.032
Egg Yolk*	0.002-0.003	0.006-0.008	0.016-0.023

* day 28 eggs

The data indicate that residues >0.01 ppm may occur in all tissue except leg muscle at the 1X feeding level, but not in egg white or yolk. At the 3 and 10X feeding levels residues >0.01 ppm are likely to be found in all tissues, egg white and yolk.

Copies of the radio-HPLC profiles for the sample extracts have been submitted. The following compound peaks are identified from these curves: pyridate, CL 9673, CL 9673-N-glucuronide and CL 9673-O-glucuronide. Numerous additional peaks appear which have not been identified. The petitioner has not quantitated the known compounds, therefore no conclusions can be drawn concerning the residues found in poultry and eggs from this feeding study. The petitioner must

quantitate the levels at which the various residue components are present. As stated in the DEB review of December 14, 1989 cited above, the nature of the residue in poultry is inadequately defined. A determination should be made of the percent of the total residue represented by the unidentified peaks. If these unknown peaks represent single compounds and are of significant quantity, they will have to be identified.

The feeding study on laying hens is inadequate. The residues which have been identified must be quantitated. In addition, data are needed on the nature of the unidentified peaks, i.e., whether these peaks represent single compounds, and the percent of the total residue which is unidentified. The nature of the residue in poultry remains inadequately defined.

OTHER CONSIDERATIONS

The current submissions present residue data reflecting radiolabelled feeding studies in lactating cows and laying hens. The petitioner has been informed previously that if meat, milk, poultry and egg tolerances are needed analytical methodology will be necessary which can successfully determine qualitatively and quantitatively the residues resulting from a "cold" feeding study. This analytical methodology should be suitable for enforcement purposes.

cc: PP#8F3603, E.Haeberer, RF, SF, Reg. Std. File, Circu (7), PMSD/ISB
RDI: Robert S. Quick, 2/15/90; Richard A. Loranger, 2/16/90
eth